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Research Article

# Evaluating the conservation effectiveness of *Ammopiptanthus nanus* (Fabaceae) under different *ex situ* strategies through multi-molecular marker analysis

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## Abstract

*Ammopiptanthus nanus* (Fabaceae), a nationally protected endangered evergreen shrub endemic to Central Asian deserts, is severely threatened by habitat fragmentation. To conserve its genetic resources, multiple *ex situ* populations have been established across China. We evaluated conservation efficacy under distinct management strategies across four *ex situ* populations (n = 114), including a near-site protected area (J), a Forestry and Grassland Administration–managed zone (L), Tazhong Botanical Garden (T), and the Forestry Academy of Sciences (K), using integrated molecular markers: expressed sequence tag–simple sequence repeats (EST-SSRs), chloroplast DNA fragments (*psbA–trnH*, *trnL–trnF*, *trnS–trnG*), and nuclear ribosomal ITS (ITS1/ITS4) sequences. The results reveal significant strategy-dependent divergence in genetic conservation efficiency, primarily driven by founder genetic composition and reproductive management protocols. Strategy (J) retained moderate chloroplast diversity but exhibited reduced nuclear diversity and high inbreeding. Strategy (L) maintained moderate nuclear diversity yet limited chloroplast variation. Strategy (T) showed the highest nuclear diversity but minimal maternal lineage preservation. Strategy (K) preserved unique maternal haplotypes and phylogenetic distinctiveness despite constrained nuclear diversity. Critically, all populations displayed high inbreeding coefficients (F > 0.404), indicating genetic bottlenecks and restricted gene flow. To ensure long-term viability, we recommend: (1) diversifying genetic foundations through the introduction of founders from multiple wild sources and expansion of population sizes to mitigate drift; (2) enhancing gene flow via periodic interpopulation transplants and facilitated distant cross-pollination, integrated with long-term monitoring of nuclear and cytoplasmic diversity.

**Key words:** *Ammopiptanthus nanus*, conservation genetics, *cp*DNA markers, endangered species, *ex situ* conservation, genetic diversity, maternal lineages

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## Introduction

Global biodiversity faces unprecedented threats from climate change, anthropogenic activities, and habitat fragmentation, accelerating species extinction rates (Pimm et al. 2014). Consequently, biodiversity conservation has become a global priority, pursued through complementary *in situ* and *ex situ* strategies (Zegeye 2017). While *in situ* conservation preserves species within their native ecosystems (Primack 2006), *ex situ* programs are critical for safeguarding taxa with severely depleted populations or degraded habitats (Li and Pritchard 2009). For endangered species, *ex situ* conservation acts as an essential genetic reservoir, enabling future reintroduction and mitigating extinction risks (Cochrane et al. 2007; O'Donnell and Sharrock 2017).

*Ammopiptanthus nanus* (Fabaceae), an endangered relict shrub endemic to Central Asian deserts, is consequently highly reliant on *ex situ* conservation. This species holds dual protection status as a National Category II (Xinjiang Forestry Department 1998) and Xinjiang Category I protected plant. Its designation as an endangered species and its unique ecological role as one of the only two evergreen broad-leaved shrubs in the Asian temperate desert (the other being *Ammopiptanthus mongolicus*) (Liu et al. 2015) affirm its high conservation priority. *A. nanus* possesses exceptional stress tolerance against drought, temperature extremes, salinity, and freezing, underpinned by specialized xeromorphic adaptations (Yu et al. 2017; Wang et al. 2023). These traits confer significant ecological value, particularly in combating desertification and stabilizing soils (Sun et al. 2021; Zhu et al. 2023), and the species also possesses documented medicinal properties (Yao et al. 2025). However, its survival is severely threatened by habitat fragmentation, intrinsically low reproductive rates, and demographic decline characterized by a scarcity of seedlings amid dominant mature individuals (Shi et al. 2018; Hao et al. 2019). Consequently, multiple *ex situ* conservation programs have been established across China to secure its genetic resources and support population recovery efforts.

The long-term success of *ex situ* conservation hinges critically on the effective preservation of genetic diversity—the cornerstone of evolutionary potential and adaptive capacity (Salgotra and Chauhan 2023). Historical population bottlenecks have already resulted in exceptionally low genetic variation within *A. nanus* (Liu et al. 2023). Therefore, rigorous assessment of the genetic integrity maintained within *ex situ* collections is paramount for formulating informed conservation plans (Khoury et al. 2010). Crucially, the efficacy of these programs depends on factors such as the genetic representativeness of founder stocks and subsequent management practices (e.g., breeding regimes), which remain largely unevaluated for this species. Molecular markers provide indispensable tools for such genetic audits. Expressed sequence tag–simple sequence repeats (EST-SSRs) offer high-resolution assessment of nuclear (biparental) genetic diversity due to their high polymorphism and reproducibility (Madhumati 2014) and have been successfully applied to analyze *ex situ* populations of *A. nanus* (Liu et al. 2023) and other endangered plants (Hou et al. 2012; Wilson et al. 2017). Complementarily, chloroplast DNA (*cp*DNA) sequences exhibiting maternal inheritance and the nuclear ribosomal internal transcribed spacer (ITS) region are powerful for tracing maternal lineages and phylogenetic distinctiveness. Integrating these markers—specifically

the *cp*DNA fragments *psbA–trnH*, *trnL–trnF*, and *trnS–trnG*, alongside ITS and EST-SSRs—enables a multidimensional evaluation of genetic conservation success. This integrated strategy has been successfully applied in species such as *Chimonobambusa utilis* (Liu et al. 2022), *Carya sinensis* (Song et al. 2024), and *Yunnanopilia longistaminea* (Yang et al. 2025).

To address this critical gap, we conducted a comprehensive genetic analysis of four *ex situ* populations of *A. nanus* that were established and managed under contrasting regimes: Tazhong Botanical Garden (T; ~1,100 m)—founded ~22 years ago, maintained through large-scale, multigenerational cultivation with intermittent wild genetic supplementation; Forestry Academy of Sciences (K; ~2,180 m)—established ~8 years ago from 3–5 wild founders, with no subsequent management or supplementation; near-site conservation (J; ~2,500 m)—a fenced, semi-natural population relying on natural regeneration; and Forestry and Grassland Administration (L; ~2,180 m)—recently established (<4 years) from 2–3 wild founders, receiving only basic horticultural care. These independently initiated programs lack standardized protocols for founder sampling or genetic management, making a comparative genetic evaluation both timely and informative. Using EST-SSRs, *cp*DNA (*psbA–trnH*, *trnL–trnF*, *trnS–trnG*), and nuclear ITS data from 114 individuals, this study aims to (1) quantify conserved nuclear and chloroplast diversity, (2) determine genetic structure and differentiation, and (3) formulate evidence-based management recommendations. This work provides the first integrated genetic assessment of *A. nanus ex situ* programs, offering direct insights for refining conservation strategies for this threatened desert relict.

## Materials and methods

### Sample collection

A total of 114 *A. nanus* individuals were sampled from four key *ex situ* populations (J, L, K, T) in Xinjiang, China, selected for their spectrum of conservation strategies and distinct management regimes and founder genetic backgrounds (Fig. 1; Table 1). Fresh leaves were collected from each sampled individual, rapidly desiccated in silica gel, and subsequently stored at −20 °C prior to DNA extraction. Precise geographic coordinates for all sampling locations were recorded using a GPS receiver and visualized using ArcGIS v10.7 (ESRI, Redlands, CA, USA).

### DNA extraction and marker amplification

Total genomic DNA was extracted from silica-dried leaf tissues using the DNAsecure Plant Kit (Tiangen Biotech, Beijing, China). Three complementary molecular marker systems were amplified: (1) nuclear EST-SSRs targeting 15 polymorphic loci, with primers sourced from Liu et al. (2023) and listed in Suppl. material 1: table S1, and their corresponding genetic diversity parameters summarized in Suppl. material 1: table S3; (2) chloroplast DNA fragments (*psbA–trnH*, *trnL–trnF*, *trnS–trnG*), using primers described by Taberlet et al. (1991) and Shaw et al. (2005) (see Suppl. material 1: table S2); and (3) the nuclear ribosomal DNA internal transcribed spacer (ITS1–ITS4) region, using universal primers developed by White et al. (1990) (Suppl. material 1: table S2). PCR amplification

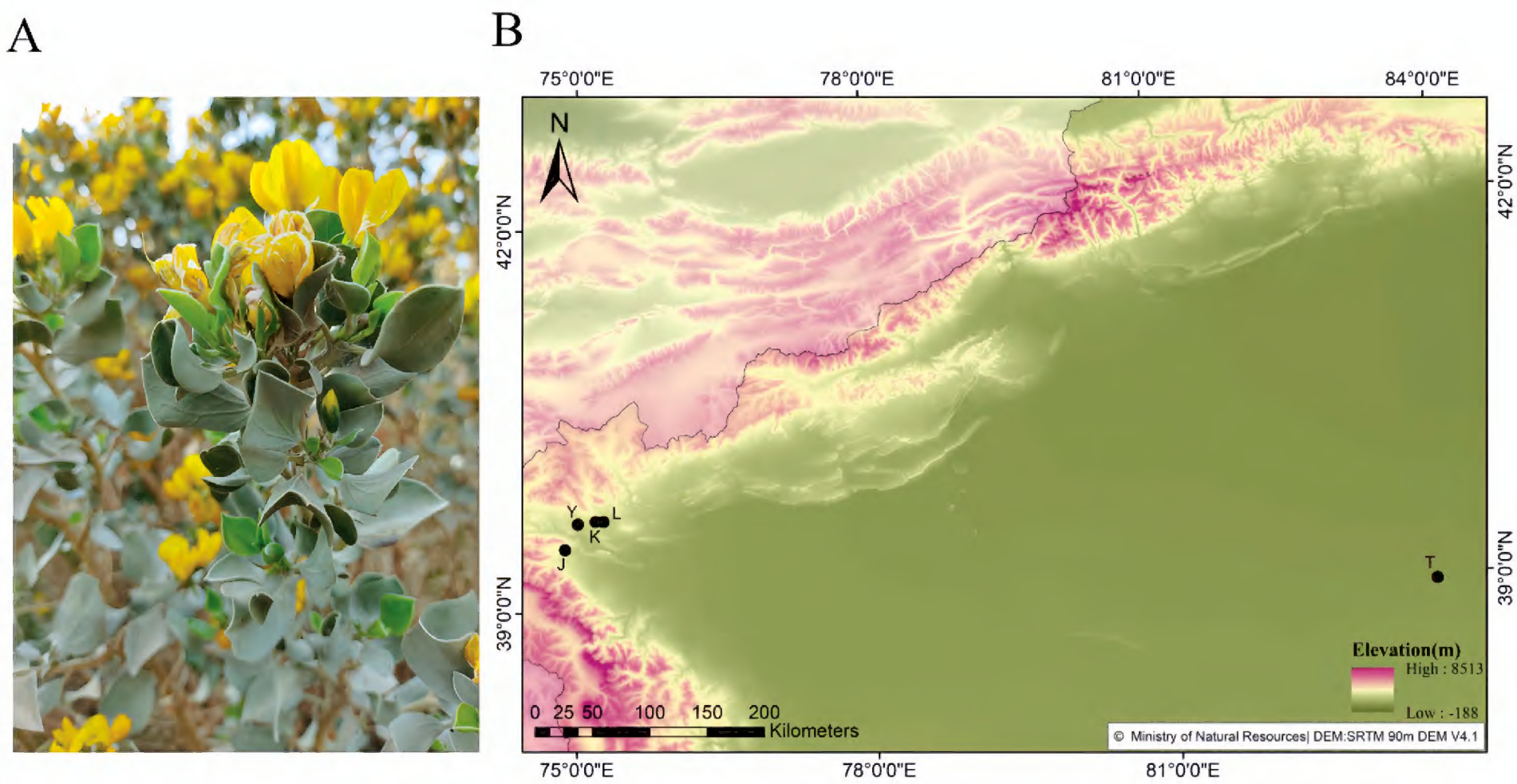



**Figure 1.** *Ammopiptanthus nanus* and its sampling geographical distribution. **A.** A flowering individual of *A. nanus* from the *ex situ* conservation population T (Tazhong Botanical Garden). This population is maintained under intensive artificial cultivation; **B.** Geographical distribution of the four *ex situ Ammopiptanthus nanus* populations (J, K, L, T) sampled in this study (total n = 114). The location of the wild population (Y) is indicated for reference. Black points denote sampling sites. See Table 1 for details.

**Table 1.** Specific geographical locations and sample sizes of different *Ammopiptanthus nanus* populations.

| Pop | N | Longitude, Latitude | Altitude | Coordinate uncertainty (m) |
|---|---|---|---|---|
| (Y) | 27 | 75°0.6'E, 39°42.0'N | 2230 | ±16 |
| (T) | 30 | 83°39.6'E, 38°58.2'N | 1100 | ±16 |
| (L) | 30 | 75°11.4'E, 39°43.2'N | 2180 | ±16 |
| (J) | 30 | 74°52.8'E, 39°30.0'N | 2500 | ±16 |
| (K) | 24 | 75°16.2'E, 39°43.2'N | 2180 | ±16 |

was performed in 25 μL reactions containing 12.5 μL Taq PCR Master Mix, 1 μL each forward and reverse primer (10 μM), 1 μL template DNA (~20 ng/μL), and 9.5 μL dd$H_2O$. Thermal cycling conditions were as follows. For EST-SSRs: initial denaturation at 94 °C for 2 min; 30 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 2 min; and a final extension at 72 °C for 5 min. For *cp*DNA and ITS: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 56 °C for 1 min, and 65 °C for 3 min; and a final extension at 72 °C for 5 min.

## EST-SSR genotyping and analysis

SSR fragment sizing was performed using GeneMarker v2.2.0 (SoftGenetics, State College, PA, USA) based on raw high-performance capillary electrophoresis (HPCE) data. Genetic diversity parameters were calculated for each population (L, T, J, K, and Y) using GenAlEx v6.5 (Peakall and Smouse 2006), including the number of alleles per locus (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected

heterozygosity (uHe), fixation index (F), Shannon's Information Index (I), and percentage of polymorphic loci (PPB). Additionally, allelic richness (Ar) was calculated using the rarefaction method (Kalinowski 2005) implemented in the R package hierfstat (Goudet 2005), standardizing all populations to the minimum sample size (n = 24). Wright's F-statistics ($F_{ST}$) and pairwise Nei's genetic distance (Nei 1972) were also calculated. Principal Coordinate Analysis (PCoA) and Analysis of Molecular Variance (AMOVA) were subsequently conducted in GenAlEx to assess genetic structure and variance partitioning.

Population genetic structure was assessed using three approaches: (1) neighbor-joining (NJ) tree, constructed in MEGA v3 (Kumar et al. 1994) based on Nei's genetic distance; (2) Bayesian clustering, implemented in STRUCTURE v2.3 (Pritchard et al. 2000) with K values from 1–10, ten independent runs per K, a burn-in period of 10,000 iterations, and 100,000 Markov Chain Monte Carlo (MCMC) replicates; and (3) optimal K determination, analyzed using Structure Selector, with results visualized using CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007) and DISTRUCT v1.1 (Rosenberg 2004).

### *cp*DNA and ITS sequence analysis

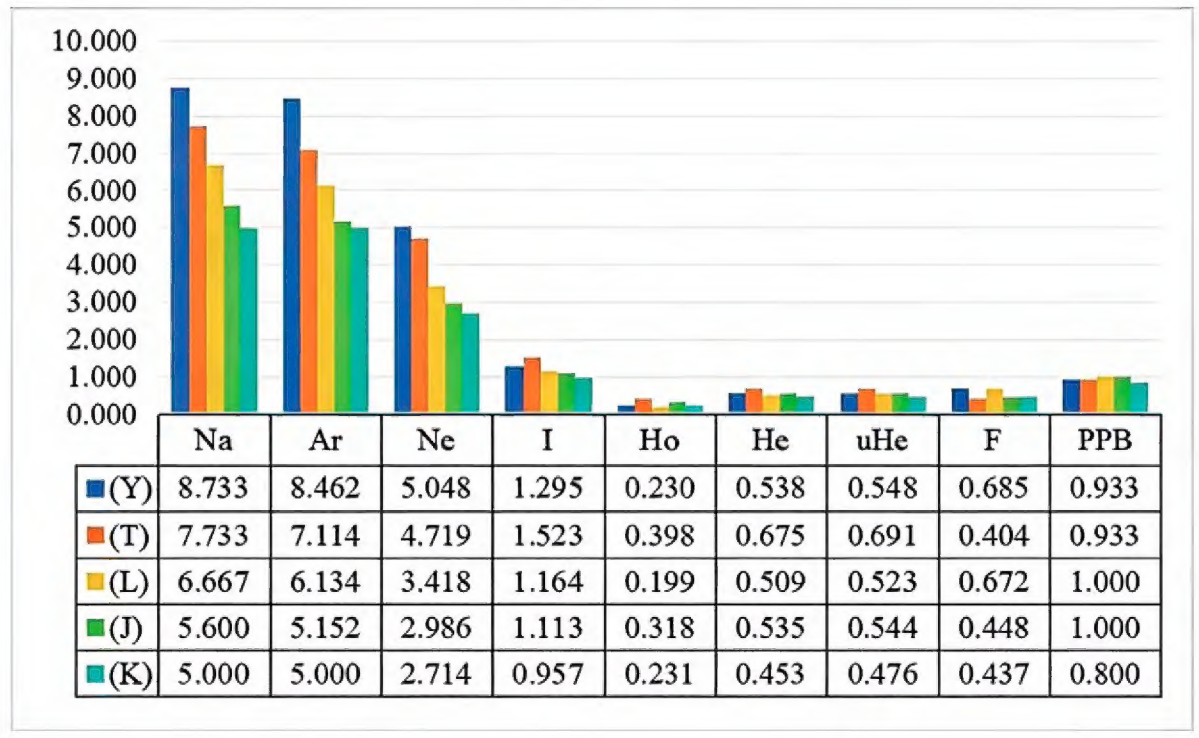
Sequences were assembled and edited in Geneious Prime v2022.0.2 (Kearse et al. 2012). The three *cp*DNA fragments (*psbA–trnH*, *trnL–trnF*, and *trnS–trnG*) were concatenated into a single sequence. Multiple sequence alignments for *cp*DNA and ITS were generated separately using the MAFFT plugin within Geneious. Haplotype analysis was conducted in DnaSP v6 (Rozas et al. 2017) to determine the number of haplotypes (H), polymorphic sites (h), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (K). TCS haplotype networks were constructed in PopART v1.7 (Leigh et al. 2015). Maximum likelihood (ML) phylogenetic trees for *cp*DNA and ITS datasets were generated using IQ-TREE v1.6.12 (Nguyen et al. 2015) with *Ammopiptanthus mongolicus* and *Sophora flavescens* as outgroups. Gene accession numbers for these outgroup species are provided in Suppl. material 1: table S4. Pairwise $F_{ST}$ and AMOVA were calculated in Arlequin v3.5 (Excoffier and Lischer 2010) to quantify population differentiation.

## Result

### Genetic diversity among populations based on SSR markers

Genetic diversity analysis across 15 EST-SSR loci revealed substantial variation among populations (Fig. 2). To enable unbiased comparison, allelic richness (Ar) was estimated via rarefaction standardized to the smallest sample size (n = 24).

The wild population (Y) possessed the highest allelic diversity (mean Na = 8.733, Ar = 8.462) but exhibited severe heterozygote deficiency (Ho = 0.230, F = 0.685). Among *ex situ* populations, T maintained the highest genetic diversity, showing maximum values for Shannon's Information Index (I = 1.523), expected heterozygosity (He = 0.675), and observed heterozygosity (Ho = 0.398), along with substantial allelic diversity (Na = 7.733, Ar = 7.114) and the lowest fixation index (F = 0.404). Populations L and J retained complete polymorphism (PPB = 1.000) but differed in diversity levels. Population L showed moderate allelic




| | Na | Ar | Ne | I | Ho | He | uHe | F | PPB |
|---|---|---|---|---|---|---|---|---|---|
| (Y) | 8.733 | 8.462 | 5.048 | 1.295 | 0.230 | 0.538 | 0.548 | 0.685 | 0.933 |
| (T) | 7.733 | 7.114 | 4.719 | 1.523 | 0.398 | 0.675 | 0.691 | 0.404 | 0.933 |
| (L) | 6.667 | 6.134 | 3.418 | 1.164 | 0.199 | 0.509 | 0.523 | 0.672 | 1.000 |
| (J) | 5.600 | 5.152 | 2.986 | 1.113 | 0.318 | 0.535 | 0.544 | 0.448 | 1.000 |
| (K) | 5.000 | 5.000 | 2.714 | 0.957 | 0.231 | 0.453 | 0.476 | 0.437 | 0.800 |

**Figure 2.** Genetic diversity parameters of *A. nanus* populations based on EST-SSR analysis. Abbreviations: Na = mean number of alleles per locus; Ne = effective number of alleles; I = Shannon's Information Index; Ho = observed heterozygosity; He = expected heterozygosity; uHe = unbiased expected heterozygosity; F = fixation index; PPB = percentage of polymorphic loci.

diversity (Na = 6.667, Ar = 6.134) coupled with the lowest overall Ho (0.199) and high inbreeding (F = 0.672), whereas population J displayed intermediate diversity parameters (Na = 5.600, Ar = 5.152, He = 0.535, Ho = 0.318, F = 0.448). Population K showed the most constrained variation, with the lowest values for most metrics (Na = 5.000, Ar = 5.000, I = 0.957, He = 0.453), reduced polymorphism (PPB = 0.800), and low heterozygosity (Ho = 0.231, F = 0.437).

All populations exhibited positive fixation indices (F = 0.404–0.685). The gradient of allelic richness (Y > T > L > J > K) confirms that population T most effectively preserved the nuclear genetic diversity of the wild source.

## Phylogenetic structure and differentiation based on SSR markers

STRUCTURE analysis indicated an optimal value of K = 5 (Fig. 3C). Beyond this overall genetic partitioning, both individual-based analyses highlighted the distinctiveness of population K. Specifically, the NJ tree revealed several subgroups (Fig. 3B) that were largely consistent with the STRUCTURE results, whereas PCoA clearly separated all individuals into two primary groups (Fig. 3A). One group exclusively contained individuals from population K, and the other comprised a cluster of all remaining individuals centered around the wild population Y. Within this larger cluster, individuals from populations Y, L, T, and J exhibited varying levels of genetic admixture in the STRUCTURE plot (Fig. 3D). Consistent across all analyses, population K was the most genetically distinct, followed by population J.

The AMOVA revealed that the majority of genetic variation in the *A. nanus* populations resided within populations (90%), with a smaller proportion occurring among populations (10%) (Table 2). This indicates that genetic diversity is primarily distributed among individuals within the same population.

Additionally, Nei's genetic distance and $F_{ST}$ values (Table 3) reflected varying degrees of genetic divergence among populations. According to Wright (1978), $F_{ST}$ values can be classified into three categories: low (0–0.05), moderate

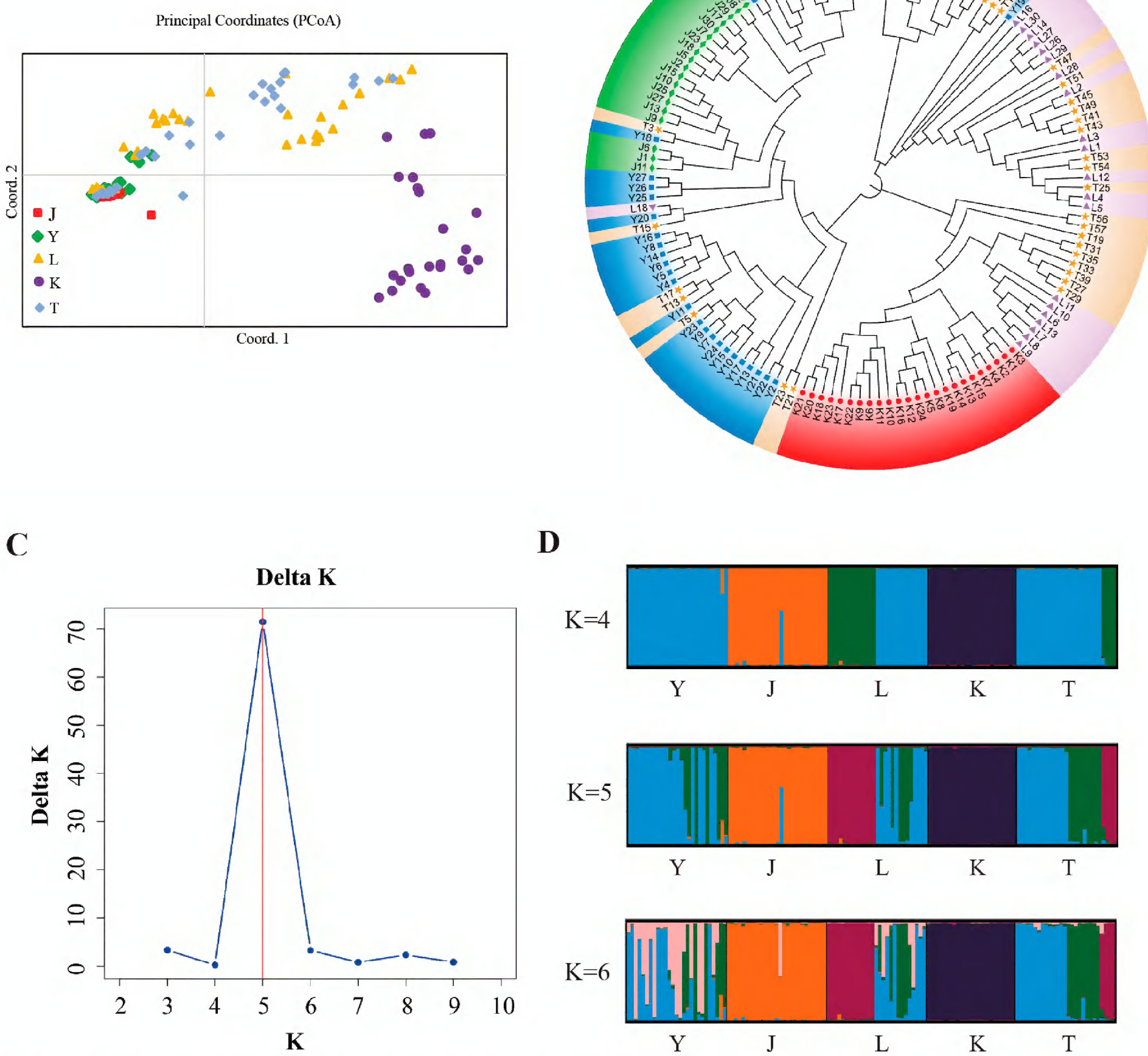



**Figure 3.** Population genetic structure analysis. **A.** Principal coordinate analysis (PCoA) plot based on genetic distance, showing the distribution of *A. nanus* samples on axes 1 and 2; **B.** Phylogenetic tree of five *A. nanus* populations based on Nei's standard genetic distance using the neighbor-joining method. Symbols: blue square (Y), green diamond (J), red circle (K), purple triangle (L), and brown star (T); **C.** Genetic structure analysis of five *A. nanus* populations based on Bayesian modeling, showing the optimal K values with a red line; **D.** Individual ancestry proportions (Q) for K = 4, K = 5, and K = 6 inferred from STRUCTURE-like analysis. Each bar represents one individual, with colors indicating genetic clusters. Population labels: (Y), (J), (L), (K), and (T).

(0.05–0.15), and high (0.15–0.25) levels of genetic differentiation (Wright 1978). Our analysis showed that population (K) exhibited the highest genetic divergence from the other populations (Y, L, T, and J), with Nei's genetic distances ranging from 1.404 to 3.739 and $F_{ST}$ values ranging from 0.283 to 0.363. In contrast, populations (Y), (L), and (T) displayed low levels of genetic differentiation, with Nei's genetic distances ranging from 0.112 to 0.288 and $F_{ST}$ values ranging from 0.047 to 0.089 (Table 3).

**Table 2.** Analysis of molecular variance (AMOVA) of *A. nanus*.

| Source of variation | *df* | SS | MS | Est. var. | % |
|---|---|---|---|---|---|
| Among populations | 4 | 208.515 | 52.129 | 0.723 | 10% |
| Among individuals within populations | 136 | 1554.709 | 11.432 | 4.831 | 66% |
| Total | 140 | 1763.224 | | 5.554 | 76% |

**Table 3.** Pairwise genetic differentiation among *A. nanus* populations: Nei's genetic distance (above diagonal) and $F_{ST}$ values (below diagonal).

| Nei genetic distance vs. $F_{ST}$ values | (Y) | (J) | (L) | (K) | (T) |
|---|---|---|---|---|---|
| **(Y)** | – | 0.744 | 0.112 | 2.999 | 0.288 |
| **(J)** | 0.186 | – | 0.977 | 1.404 | 1.129 |
| **(L)** | 0.047 | 0.224 | – | 3.739 | 0.256 |
| **(K)** | 0.347 | 0.287 | 0.363 | – | 3.296 |
| **(T)** | 0.089 | 0.191 | 0.089 | 0.283 | – |

## Haplotype identification and genetic diversity among populations based on *cp*DNA and ITS sequences

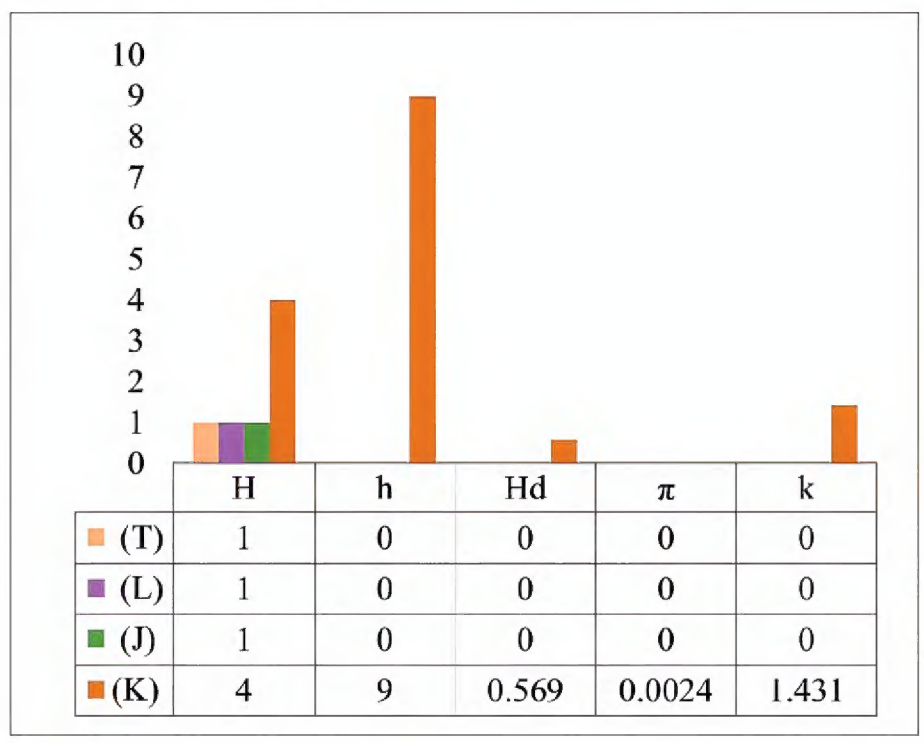
We identified varying numbers of haplotypes across different *ex situ* conservation populations (L/T/J/K) based on both *cp*DNA and ITS sequences. Among these populations, the population K demonstrated the greatest number of haplotypes, comprising four haplotypes identified through ITS sequences and five haplotypes identified through *cp*DNA. Additionally, it exhibited the highest level of genetic diversity compared to the other populations (ITS/*cp*DNA: h = 4/9, Hd = 0.569/0.616, π = 0.0024/0.0013, K = 1.431/1.996). In contrast, the population (T) exhibited the lowest level of genetic diversity, with only one haplotype detected. The populations (J) and (L) both detected only a single haplotype from ITS sequences; however, each demonstrated polymorphism in their *cp*DNA (J/L: H = 3/2, h = 4/1). The genetic diversity observed in population J (Hd = 0.239, π = 0.00022, K = 0.333) was significantly higher than that in population L (Hd = 0.087, π = 0.0006, K = 0.087) (Fig. 4).

The AMOVA based on *cp*DNA and ITS sequences similarly indicated that a higher proportion of genetic variation occurred within populations (ITS/*cp*DNA: 68%/71%) than among populations (ITS/*cp*DNA: 32%/29%) (Tables 4, 5). This again suggests that genetic variation is primarily found within rather than between populations. Moreover, the $F_{ST}$ values for different pairwise populations between the ITS sequence and *cp*DNA showed comparable results. Notably, the population (K) displays a higher degree of genetic differentiation from the other populations (0.278–0.452), while no significant genetic differentiation is observed among the remaining three populations (L, T, and J) (Fig. 5).

## Phylogenetic structure based on ITS sequences and *cp*DNA

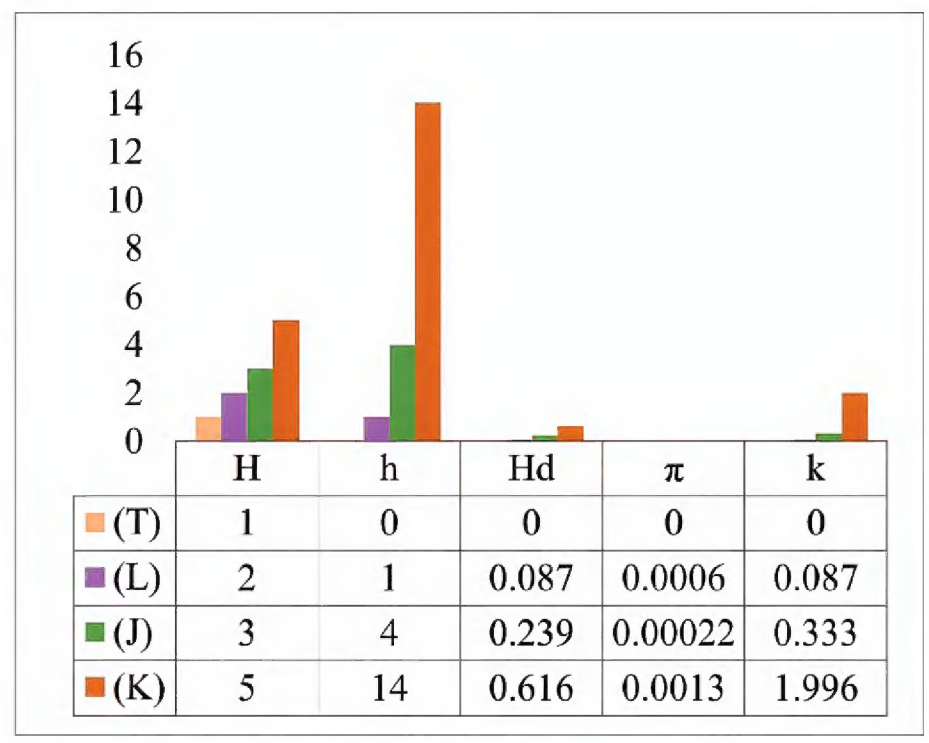
The phylogenetic tree constructed using maximum likelihood (ML) based on ITS sequences and *cp*DNA revealed similar topological structures, indicating that all individuals of *A. nanus* form a monophyletic clade. Within this clade, most individuals from population K clustered distinctly. In contrast, individuals

A




| | H | h | Hd | π | k |
|---|---|---|---|---|---|
| (T) | 1 | 0 | 0 | 0 | 0 |
| (L) | 1 | 0 | 0 | 0 | 0 |
| (J) | 1 | 0 | 0 | 0 | 0 |
| (K) | 4 | 9 | 0.569 | 0.0024 | 1.431 |

B




| | H | h | Hd | π | k |
|---|---|---|---|---|---|
| (T) | 1 | 0 | 0 | 0 | 0 |
| (L) | 2 | 1 | 0.087 | 0.0006 | 0.087 |
| (J) | 3 | 4 | 0.239 | 0.00022 | 0.333 |
| (K) | 5 | 14 | 0.616 | 0.0013 | 1.996 |

**Figure 4. A.** ITS genetic diversity parameter values; **B.** *cp*DNA genetic diversity parameter values.

**Table 4.** Analysis of molecular variance (AMOVA) based on ITS sequences.

| Source of variation | d.f. | Sum of squares | Variance components | % |
|---|---|---|---|---|
| Among populations | 3 | 6.566 | 0.8023 Va | 32% |
| Within populations | 97 | 16.609 | 0.17122 Vb | 68% |
| Total | 100 | 23.175 | 0.25145 | 100% |

**Table 5.** Analysis of molecular variance (AMOVA) based on *cp*DNA sequences.

| Source of variation | d.f. | Sum of squares | Variance components | % |
|---|---|---|---|---|
| Among populations | 3 | 23.101 | 0.27904 Va | 29% |
| Within populations | 97 | 66.225 | 0.68273 Vb | 71% |
| Total | 100 | 89.326 | 0.96177 | 100% |

from populations J, L, and T did not exhibit distinct lineage structures and were mixed across various branches (Fig. 6).

The haplotype networks based on ITS sequences and *cp*DNA both revealed a star-like pattern. H1 (ITS) and H2 (*cp*DNA) were the most common haplotypes, shared across all populations. Population K possessed the most complex network structure, with several private haplotypes (ITS: H2, H3, H4; *cp*DNA: H4, H5, H6, H7). In contrast, population T contained no private haplotypes. Population J included one private *cp*DNA haplotype (H1), and *cp*DNA haplotype H3 was shared exclusively by populations J and L (Fig. 7).

## Discussion

### Genetic diversity assessment and the evolutionary underpinnings of high inbreeding in *A. nanus*

Genetic diversity underpins species adaptability and long-term persistence (Ellegren and Galtier 2016). Our EST-SSR data, particularly the rarefaction-based allelic richness (Ar), confirm that the *ex situ* populations of *A. nanus* (L, T, J, and K) harbor reduced nuclear diversity compared to the wild population (Y). The

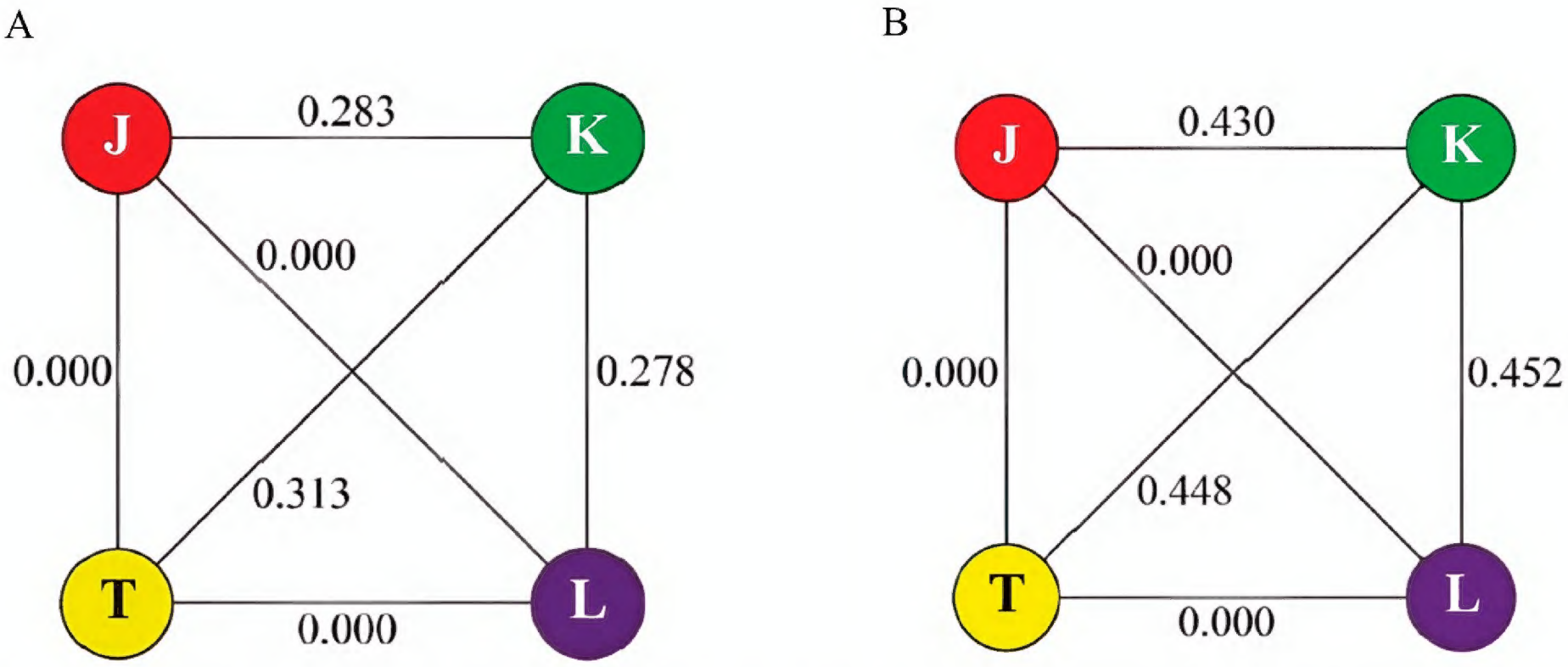



**Figure 5.** Pairwise genetic differentiation coefficients among four *A. nanus* populations: $F_{ST}$ values. **A.** Based on ITS sequences; **B.** Based on *cp*DNA.

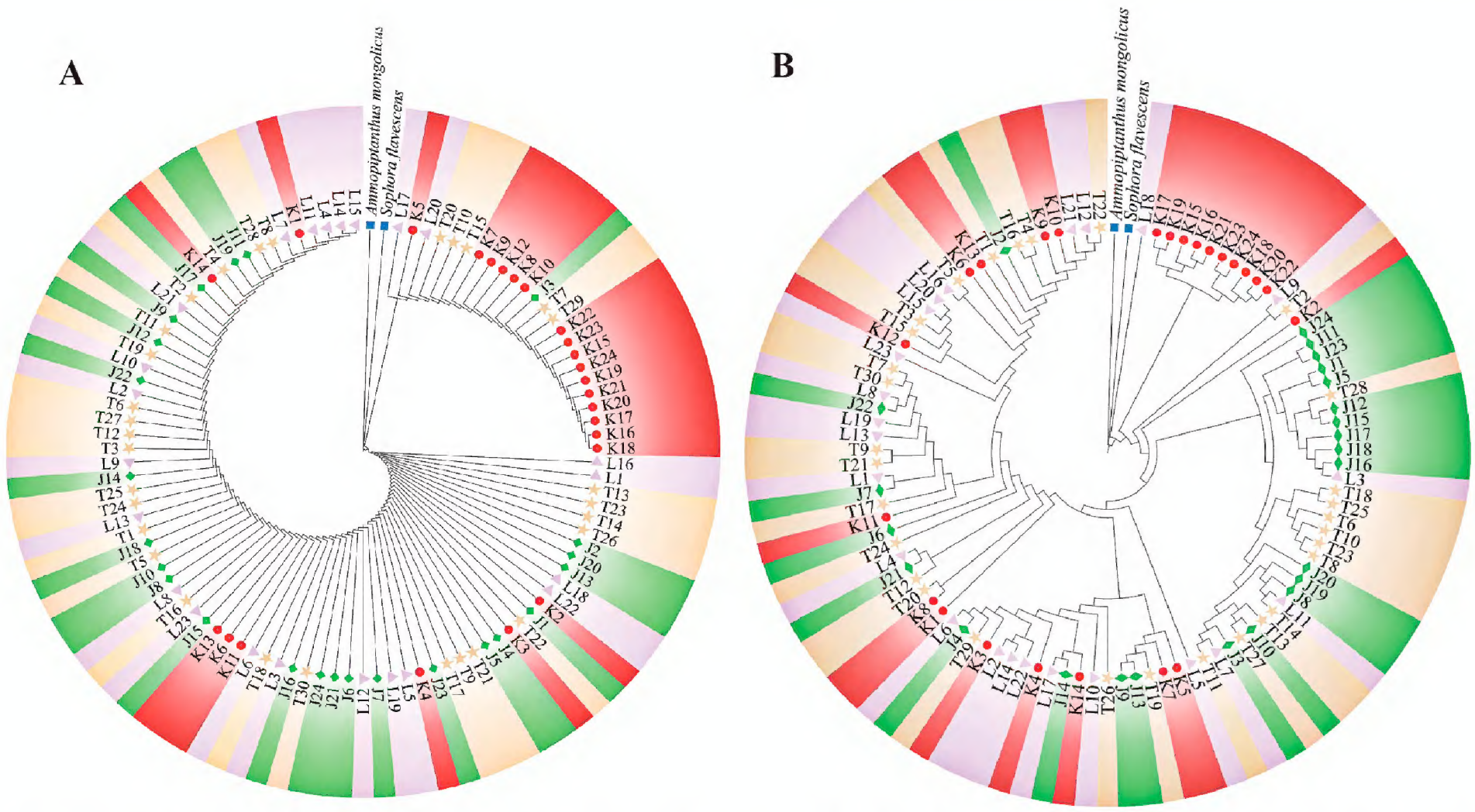



**Figure 6.** Maximum likelihood phylogenetic tree of *A. nanus* based on ITS and *cp*DNA. **A.** ITS-based tree; **B.** *cp*DNA-based tree. Symbols: green diamond (J), red circle (K), purple triangle (L), brown star (T), blue square (*Ammopiptanthus mongolicus*; *Sophora flavescens*).

observed gradient of declining allelic richness (Y > T > L > J > K) (Fig. 2) is consistent with the genetic erosion expected from founder effects and drift during artificial propagation (Nei et al. 1975; Witzenberger and Hochkirch 2011). Notably, the pervasively high inbreeding coefficients (F) across all populations—including the wild source (F = 0.685)—point to causative factors beyond *ex situ* management, rooted instead in the species' intrinsic reproductive ecology (Eckert et al. 2010).

The observed genome-wide heterozygosity deficit (He > Ho) provides a robust genetic signature of systematic non-random mating. We hypothesize two

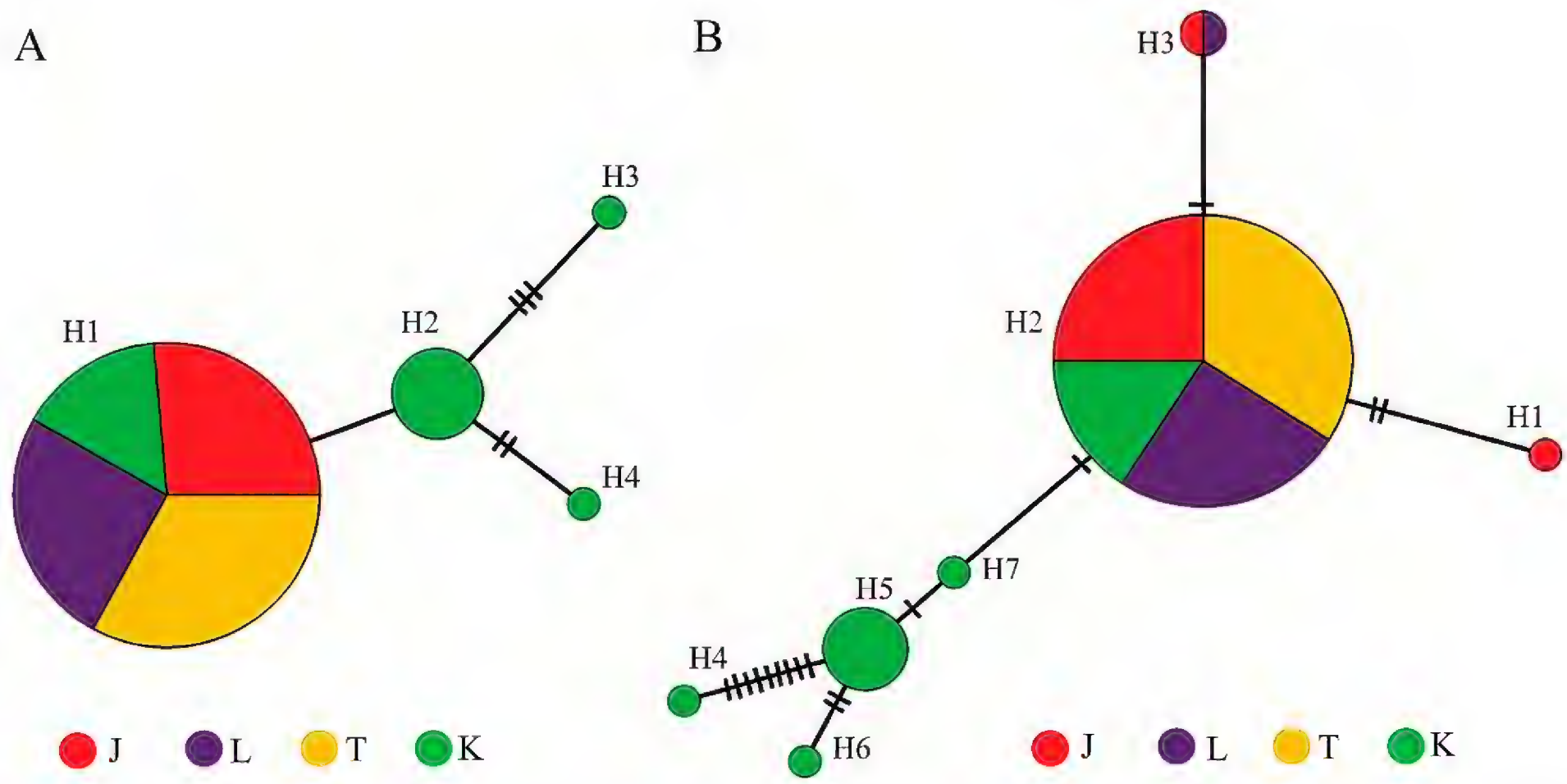



**Figure 7.** Haplotype networks of *A. nanus* based on ITS and *cp*DNA. **A.** ITS-based network; **B.** *cp*DNA-based network. Color key: green (K), red (J), purple (L), and yellow (T).

interrelated mechanisms, both inherent to the life history of a long-lived perennial shrub in fragmented habitats. First, self-compatibility and prevalent geitonogamous selfing represent a likely primary mechanism (Aguilar et al. 2008 ; Llorens et al. 2012). In such systems, insect-mediated pollination often leads to substantial within-plant fertilization, an effect amplified in sparse populations where pollinator movements are restricted (Zhang and Ye 2011; Hu et al. 2013). Second, biparental inbreeding, driven by fine-scale spatial genetic structure (SGS) (Born et al. 2008), is equally critical. Limited seed dispersal results in the spatial clustering of related individuals, meaning that local mating effectively occurs among relatives (Nathan and Muller-Landau 2000), thereby generating an excess of homozygosity detectable as high F values. Of paramount importance is the fact that all *ex situ* populations were established from seed, which definitively excludes clonal propagation as a contributing factor and underscores that mating system dynamics are the principal driver.

In this light, the extremely high F value in the wild population Y is not an anomaly but rather the expected genetic signature of its reproductive strategy operating under severe habitat fragmentation. While *ex situ* populations generally inherited this genetic background, the degree of inbreeding varies with management history. Population T, despite maintaining a high fixation index (F = 0.404), exhibits a lower value than the wild population (F = 0.685) and retains the highest allelic richness (Ar = 7.114) among conservation sites, suggesting it effectively captured a significant portion of the wild gene pool. Conversely, population K, exhibiting the lowest allelic richness (Ar = 5.000), epitomizes an extreme case of genetic drift following a particularly severe founding bottleneck. In contrast, the *cp*DNA and ITS datasets revealed a divergent pattern, with several *ex situ* populations—notably K—retaining high haplotype diversity (Fig. 4). This pronounced cytonuclear discordance (i.e., incongruent patterns of genetic variation between nuclear and organellar genomes) should not be viewed as contradictory but rather as an informative outcome of the differential

evolutionary pressures acting on genomes with distinct modes of inheritance (Lee-Yaw et al. 2019). EST-SSRs, being biparentally inherited and recombining nuclear markers, are highly sensitive to contemporary demographic perturbations such as bottlenecks and inbreeding. Conversely, the haploid, maternally inherited *cp*DNA is not subject to dilution through pollen flow (Day and Ellis 1984; Varshney et al. 2005) and thus retains a strong signal of historical maternal lineage diversity (Petit et al. 2005), largely impervious to the recent mating dynamics that rapidly reshape nuclear homozygosity. Consequently, the high *cp*DNA diversity in population K signifies that its founding cohort was derived from multiple, distinct maternal lines, thereby preserving ancient polymorphisms that have persisted despite the severe erosion of nuclear diversity (Ar). Population T presents a contrasting scenario: its single *cp*DNA haplotype coupled with higher nuclear diversity suggests that a genetically diverse pollen pool, or multiple paternal founders, introduced nuclear variation through recombination, while all propagated seeds originated from one or a few maternal sources. These contrasting patterns between population K (high *cp*DNA diversity, low nuclear Ar) and T (low *cp*DNA diversity, high nuclear Ar) underscore the critical importance of founder sampling strategy: capturing multiple maternal lineages preserves organellar history, while ensuring a diverse pool of paternal contributors is essential for maintaining nuclear genetic variation in *ex situ* collections.

In conclusion, the high inbreeding coefficients observed across the *A. nanus* metapopulation are best explained by the interaction between a self-compatible mating system and population substructure, a situation markedly intensified by habitat fragmentation. The apparent conflict between marker systems is resolved by recognizing their complementary temporal perspectives: SSR markers illuminate the consequences of recent reproductive ecology and genetic drift, whereas *cp*DNA/ITS data preserve the legacy of historical maternal lineage diversity (Lee-Yaw et al. 2019). Embracing this integrated, multi-marker viewpoint is therefore indispensable for designing conservation strategies that effectively capture and sustain the species' full evolutionary potential (Nielsen et al. 2020).

### Genetic structure and divergence in *A. nanus*

The apparent cytonuclear discordance discussed above necessitates an integrated interpretation of the genetic structure in *A. nanus*. Rather than invalidating any single dataset, the conflicting signals from nuclear and chloroplast markers, when reconciled, provide a more nuanced, multi-layered understanding of population divergence and origins. Genetic structure reflects the spatial organization of genetic variation within a species, shaped by processes such as geographical isolation, human disturbance, and natural selection (Jacquard 2012). Understanding these patterns is essential for reconstructing evolutionary history and designing effective conservation strategies (Loveless and Hamrick 1984). Our multi-marker analyses consistently revealed pronounced genetic structure and divergence among the *ex situ* populations of *A. nanus*. These findings align with earlier studies based on similar approaches (Su et al. 2016; Liu et al. 2025), supporting the view that this species possesses a complex evolutionary background, with populations originating from distinct genetic lineages.

Notably, population K exhibited strong genetic differentiation from all other groups ($F_{ST}$: 0.278–0.452; Table 3) and was consistently placed in a distinct cluster by phylogenetic reconstructions (NJ/ML; Fig. 3B, Fig. 6), PCoA (Fig. 3A), and Bayesian structure analyses (Fig. 3D), all of which suggest it was founded from genetically distinct ancestors.

In contrast, populations J, L, and T formed a cohesive group in analyses based on *cp*DNA and ITS (Figs 5, 7), indicating they likely share a common maternal origin. However, nuclear SSRs revealed a more complex pattern: while J is geographically adjacent to the wild population Y, it showed moderate genetic divergence from Y, L, and T ($F_{ST}$: 0.186–0.191; Table 3) and a distinct genetic composition in STRUCTURE output (Fig. 3D). This pattern is indicative of habitat fragmentation (Fahrig 2003), which can promote genetic divergence even between nearby stands. Interestingly, despite considerable geographic separation between populations L and T, they retained high genetic similarity ($F_{ST}$ = 0.089), implying that shared founder sources have overridden the effects of spatial isolation in the *ex situ* context.

### Integrated conservation strategy of *A. nanus*

*Ex situ* conservation serves as a pivotal strategy for safeguarding rare species, utilizing controlled environments to preserve genetic material and facilitate reproduction, thereby supporting population recovery and long-term sustainability (Reed et al. 2011). Our findings affirm the potential of this approach, as demonstrated by the considerable nuclear diversity retained in population T (I = 1.523) and the rich maternal lineage diversity preserved in population K (H = 5). Nevertheless, such programs are often compromised by founder effects and inbreeding depression, which can substantially erode genetic variation and adaptive potential (Bouzat 2010). These issues are clearly reflected in the four *ex situ* populations of *A. nanus*, all of which exhibit high inbreeding coefficients and limited inter-population gene flow. Furthermore, the genetic foundations of these populations appear to originate from relatively narrow sources. Thus, our comprehensive genetic evaluation highlights the pressing need to optimize current conservation protocols. We propose a threefold strategy to address these challenges: first, augmenting genetic diversity by introducing new founders from multiple, genetically distinct wild sources; second, promoting gene flow among *ex situ* sites through assisted cross-pollination and coordinated breeding; and third, establishing long-term genetic monitoring programs incorporating periodic assessment of both nuclear and chloroplast genomic diversity. Through these coordinated actions, the evolutionary resilience of *A. nanus* can be better maintained, enhancing its capacity to respond to environmental change and ensuring the continued success of *ex situ* conservation initiatives.

## Conclusion

This study confirms that the long-term genetic integrity of *ex situ* populations of *A. nanus* is predominantly determined by the genetic diversity and distinctness of the founding stocks, coupled with post-establishment breeding management. To sustain evolutionary resilience, we propose an integrated strategy focusing on three actionable priorities: (1) establishing new populations

with genetically diverse and distinct founders; (2) implementing assisted gene flow among existing populations; and (3) maintaining a centralized germplasm registry supported by systematic genetic monitoring. These coordinated actions are imperative to safeguard the species' adaptive potential under ongoing environmental change.

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## Additional information

### Conflict of interest

The authors declare no conflicts of interest.

### Ethical statement

No ethical statement was reported.

### Use of AI

No use of AI was reported.

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### Author contributions

Wei Shi and Jiancheng Wang: Conceptualization (equal); Funding acquisition (equal); Zhongchen Lu: Data curation; Jingdian Liu: Formal analysis; Xiyong Wang: Investigation; Supervision; Daoyuan Zhang: Methodology; Project administration; Lingling Ma: Writing – original draft (lead); Writing — review and editing (lead). All authors have read and agreed to the published version of the manuscript.

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### Data availability

The molecular datasets generated during this study, including EST-SSR genotypes, ITS, and *cp*DNA sequences, will be deposited in the Dryad repository upon acceptance of this manuscript, and the persistent identifier (DOI) will be provided in the final published version. The detailed specimen collection records (species occurrence dataset) are not available for public deposition. This is because the precise geolocation metadata for individual samples were managed by former team members and are no longer retrievable following their

graduation. However, the key population-level geographic information (site codes, sample sizes, and approximate coordinates) is provided in Table 1 of this manuscript to ensure the interpretability and reproducibility of the study.

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## Supplementary material 1

### Genomic, molecular markers, genetic diversity

Authors: Lingling Ma, Wei Shi

Data type: pdf

Explanation note: **table S1.** EST-SSR primer sequences for *A. nanus*. This table provides the detailed sequences of the EST-SSR primers used in this study; **table S2.** Primer sequences for ITS and *cp*DNA regions in *A. nanus*. This table lists the primer sequences for the nuclear ITS (ITS1–4) and chloroplast DNA (*psbA–trnH*, *trnL–trnF*, *trnS–trnG*) regions used for molecular analysis; **table S3.** Genetic diversity of 15 EST-SSR primers in *A. nanus*. This table summarizes the genetic diversity parameters, including allele numbers, heterozygosity, and polymorphism information content, for each of the 15 EST-SSR primers; **table S4.** GenBank accession numbers for outgroup species. This table provides the GenBank accession numbers for the ITS and *cp*DNA (*psbA–trnH*, *trnL–trnF*, *trnS–trnG*) sequences of the outgroup species, *Ammopiptanthus nanus*, *Ammopiptanthus mongolicus*, and *Sophora flavescens*.

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